Amendments to the Specification:

Please insert the following title and paragraph in the specification at page 1, line 3, before the "Field of the Invention":

Cross-Reference to Related Applications

a1 This application claims the benefit of U.S.S.N. 60/233,401, filed September 18, 2000.

Please delete the paragraph at page 5, line 22 through page 6, line 17 and replace it with the following amended paragraph:

The cultured connective tissue construct comprises connective tissue cells bound by an extracellular matrix, primarily of collagen. The extracellular matrix aspect of the construct may vary in organization and composition and still qualify as a connective tissue construct for use in the method of the invention. The connective tissue construct may be a contracted collagen lattice containing fibroblasts as described by Bell in U.S. Patent No. 4,485,096 or by Kemp, et al. in U.S. Patent No. 5,536,656 where the contracted collagen lattice is disposed on an acellular collagen gel, the disclosures of which is incorporated herein be reference. Another construct for use in the method is a a2 bioengineered tissue ~~constructs~~construct of cultured cells and endogenously produced extracellular matrix components without the requirement of exogenous matrix components or network support or scaffold members such as those described in PCT Publication No. WO 00/29553 to Murphy, et al., the disclosure of which is incorporated herein by reference. Connective tissue constructs such as those that incorporate a synthetic or bioresorbable mesh member having cultured fibroblasts attached [[end]]and enveloping it with endogenously produced matrix such as those described by U.S. Patent Numbers 5,580,781, 5,443,950, 5,266,480, 5,032,508, 4,963,489 to Naughton, et al. may also be used, a fibrous collagen matrix containing cultured cells therein as described by U.S. Patent Numbers 4,505,266 and 4,280,954 to Yannas, et al. Xenogeneic materials such as de-epidermalized and decellularized dermis, and other flat sheet tissues that have been cleaned of antigenic determinants and cellular debris

q2

can be used as a matrix component that is cultured with nonallogeneic cells to repopulate the construct.

Please delete the paragraph at page 8, line 14 through page 9, line 11 and replace it with the following amended paragraph:

While collagen is the most preferred extracellular matrix composition for use in the production of skin equivalents that produce and secrete cytokines to condition the culture media, other extracellular matrix components may be used. These extracellular matrix components may be used alone or, preferably, be included with the collagen to mimic native dermal matrix. These extracellular matrix components may include: other collagens, both fibrillar and non-fibrillar collagen from the collagen family such as collagen types II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, other matrix proteins that may include, but are not limited to elastin, proteoglycans such as decorin or biglycan, or glycoproteins such as tenascin, vitronectin, fibronectin, laminin, thrombospondin I, and glycosaminoglycans (GAG) such as hyaluronic acid (HA). The dermal matrix may vary in composition and ~~structure. Collagen~~structure: collagen sponges, biocompatible, bioremodelable, decellularized dermis, or collagen gels. Rather than provide extracellular matrix components to the dermal cells, they can be cultured on biodegradable mesh members (such as nylon or polygalactin (PGA)) to provide a culture support and cultured to produce extracellular matrix until the cells and their matrix ~~envelope~~envelop the support. In the preferred embodiment, the cultured connective tissue construct is a contracted collagen gel, contracted by fibroblasts such as those described in U.S. Patent No. 4,485,096 to Bell, incorporated herein by reference. In [[a]] another preferred embodiment, the contracted collagen gel is disposed on a bulk acellular collagen layer on a porous membrane to anchor the gel to the membrane and to prevent excessive radial contraction of the gel. Methods for incorporating a bulk acellular collagen layer are described in U.S. Patent No. 5,536,656 to Kemp, et al., and are incorporated herein by reference.

q3

Please delete the paragraph at page 10, line 13 through page 11, line 7 and replace it with the following amended paragraph:

In one alternative embodiment of the present invention, an acellular collagen gel is formed on the culture substrate prior to the formation of the connective tissue construct to provide an anchoring means to the substrate. In some cases, the substrate requires this anchoring means and in other cases, it is not needed. When the acellular collagen gel component is utilized in the fabrication of the cultured connective tissue construct, it has been found that the ratio of the volume of the casting mixture for the tissue equivalent to the volume of the casting mixture for the acellular, hydrated collagen gel has an effect upon cell viability and differentiation. Useful ratios, volume to volume (v/v), of tissue equivalent casting mixture to collagen gel casting mixture are about 3:1 to 1:3. A preferred ratio wherein the cell concentration in the collagen lattice is at about 2.5 x 104 cells/ml is 3:1. The acellular, hydrated collagen gel 25 is prepared from a collagen composition comprising collagen at about 0.5 to 2.0 mg/ml, preferably about 0.9 to 1.1 mg/ml and nutrient media. This collagen composition is added to the inner container 20 and maintained under conditions which permit the collagen composition to set and form an acellular, hydrated collagen gel of suitable dimensions, typically about 1 to 5 mm thick, a preferred thickness range being about 2 mm to about 3 mm. An acellular, hydrated collagen gel 25 is preferably thick enough so that a portion remains acellular as cells migrate from the tissue equivalent into an acellular, hydrated collagen gel and thin enough so that the tissue equivalent is not undesirably removed from the nutrient source provided in outer container 10.

Please delete the paragraph at page 17, lines 16-18 and replace it with the following amended paragraph:

Experimental animals are pre-anesthetized with Telazol and atropine and intubated. [[The]]They are placed on inhalation gas of isoflurane and oxygen and kept in surgical plane of anesthesia. They are also administered an antibiotic.

Please delete the paragraph at page 17, line 19 through page 18, line 4 and replace it with the following amended paragraph:

Defects in the discs are created by making a 5 x 10 mm incision in the annulus followed by a standard discotomy with equal nuclear removal at each space. A total of three discs are operated on per pig. Two sites are treated with cultured connective tissue constructs with GFP labeled fibroblasts and the remaining site serves as a control. To apply the connective tissue construct, it is first trimmed into three or four smaller pieces and then inserted into the annular hole ~~opening~~opening. Two animals are euthanized on each of weeks 2, 4, and 6 and the surgical sites are removed. The discs are placed in formalin and then 70% ethanol prior to histological processing. The discs are serially sectioned and examined under fluorescence for evidence of GFP labeled fibroblasts.

Please delete the paragraph at page 18, lines 10-11 and replace it with the following amended paragraph:

Six young pigs of either sex up to 50 kg are housed individually for a minimum of two days prior to surgery while fed with standard pig chow.

Please delete the paragraph at page 18, line 18 through page 19, line 3 and replace it with the following amended paragraph:

Through the hole made in the annulus fibrosis, the intervertebral space is opened and the disc is removed, restricted to the anterior and middle third portion. The intervertebral disc spacer comprising Dacron mesh and hydrogel is placed into the thoracic cavity by passing it through the hole in the annulus fibrosis. The good position of the implant is ascertained using radiologic procedures and then the spacer is then fixed into place. The cultured connective tissue construct is then applied to the annular opening by first trimming the construct to the size of the annular hole opening and then sutured to the tissue surrounding the opening of the space using resorbable sutures. While all three sites are provided with an intervertebral disc spacer, two sites are

G8

treated with cultured connective tissue constructs and the remaining site ~~to serve~~serves as a control.

Please delete the paragraph at page 20, lines 15-23 and replace it with the following amended paragraph:

G9

Microscopy revealed clear evidence of implanted connective tissue construct remnants, including viable fluorescent fibroblasts, in several of the treated annuli from the two animal groups euthanized at two and four weeks. There was also identifiable remodeling of the connective tissue construct remnants by the host tissue. The implanted defects showed less inflammation and more advanced healing than controls at all time points. The implanted defects had cartilaginous tissue bridging the opening, whereas the control defects still had a significant amount of fibrotic tissue. The results from this feasibility study indicate that the implanted pig connective tissue construct [[were]]was biocompatible to the host tissue, can persist up to 4 weeks, and enhance reparative activities of the annulus by 6 weeks.